

THE ROLE OF ROOT-KNOT NEMATODE
IN PREDISPOSING CERTAIN
TOMATO VARIETIES TO
SOUTHERN BACTERIAL WILT

By

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

June, 1965

TO THE PEOPLE OF LIBYA AND TO THE MEMORY
OF MY FATHER THIS WORK IS DEDICATED

ACKNOWLEDGMENTS

The author wishes to thank the chairman of his supervisory committee, Dr. Phares Decker, for his advice and guidance during the course of this work. The author is deeply indebted to the members of the committee, Drs. D. A. Roberts and V. G. Perry, for their unlimited cooperation, valuable suggestions throughout the course of this investigation, and for their help in the preparation of the manuscript. Sincere appreciation is expressed to Dr. L. W. Ziegler, a member of the committee, for his help and encouragement.

The author gratefully acknowledges Dr. W. R. Henderson, Department of Horticultural Sciences, North Carolina State College, Raleigh, North Carolina, for supplying the seeds of the "MO.P" tomato line. The author also wishes to thank Dr. E. A. Brandt, former Head Statistician, Agricultural Experimental Station, University of Florida, Gainesville, Florida, for his suggestions and assistance. Special thanks are extended to Mrs. Helen Balyeat for typing the manuscript.

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INTRODUCTION

Plant parasitic nematodes have been frequently associated with disease complexes involving other soil-borne plant pathogens. Christie (20) grouped these etiological associations into two groups. One is of an obligatory nature whereby one member is dependent on another or directly influenced by it. The second type is strictly fortuitous, in which each member acts independently and is not influenced by the associated pathogen. The latter is exemplified by the frequent association of nematode injury with secondary invaders. In the obligate association the nematode may act as (a) an inoculating agent or vector, (b) a complementary etiological factor in the disease syndrome, (c) a synergistic or (d) an antagonistic agent. Review of the literature reveals the existence of nematode association with fungal, bacterial and viral pathogens. Several review articles concerning interaction between nematodes and other pathogens have been published (20, 29, 42, 94, 96, 100, 134).

REVIEW OF THE LITERATURE

Virus-nematode interactions

Plant parasitic nematodes have been reported as vectors of several soil-borne plant viruses (100). Nematodes act as inoculating and possibly survival agents for plant viruses. Ectoparasitic species of Xiphinema, Trichodorus, and Longidorus are widely accepted as vectors

of viruses. Interaction between Meloidogyne incognita acrita and tobacco ring spot virus (TRSV) has been investigated (104, 105). Presence of the virus and the nematode significantly reduced length of soybean roots in comparison to infection by either pathogen alone. Furthermore, in virus-infected plants the lysigenomata (giant cells) had more clumping, greater number of nuclei in vicinity of the nematode's heads, and greater amount of protoplasm than those in virus-free plants (105). The same authors, in contrast, found that the presence of the virus in nematode-susceptible or resistant soybean varieties did not influence the reactions of either variety to infection by the nematode (104). Although the authors did not explain such contradictory results, differences in parasitic races of the nematode and strains of the virus might have been contributing factors.

Fungal-virus interactions have also been reported. Infection by cucumber mosaic virus (CMV) significantly increased susceptibility of cucumber to post-emergence damping off caused by Rhizoctonia solani (7). Synergistic interaction was greatest when low inoculum concentration of the fungus and high inoculum level of the virus were used. These results suggested that movement of materials from the roots to the CMV-infected cotyledons, which showed increased respirations 48 hours after inoculation, increased susceptibility to the fungus. Similarly, infection by pea mosaic virus (PMV), pea enation mosaic virus (PEMV), alfalfa mosaic virus (AMV), or bean yellow mosaic virus (BYMV) significantly increased susceptibility of peas to Aphanomyces euteiches and Fusarium solani f. pisi (26). Presence of either fungus did not alter symptom expression of any of the viruses tested. Clover yellow mosaic virus

(CYMV) and white clover mosaic virus (WCMV) significantly increased severity of root rot of red clover caused by either F. oxysporum, F. roseum, or Tetracoccosporium parianum (132). Fungi, alone, readily penetrated clover roots but were only mildly pathogenic.

Fungus-nematode interactions

Damping off diseases.--Feldmesser and Fedder (28) found that the combination of Radopholus similis with either Rhizoctonia solani or Fusarium solani resulted in a significant increase in root damage of grapefruit seedlings in comparison to that produced by each pathogen alone. Similarly, reduction in root growth of sweet orange seedlings caused by Tylenchulus semipenetrans and F. solani was greater than that produced by either the fungus or the nematode (129). Halpin (38) reported that infection by Meloidogyne incognita or M. incognita acrita significantly increased severity of root damage of white clover caused by R. solani, F. roseum or Nigrospora sp. Reynolds and Hanson (101) demonstrated a close association between severity of damping off of cotton caused by R. solani and root knot severity caused by M. incognita acrita. Soil fumigation with nematocides decreased the nematode population and effectively controlled damping off of cotton. Meloidogyne javanica and M. hapla markedly increased post-emergence damping off of soybeans caused by R. solani (124). Similarly, both nematodes interacted synergistically with virulent, but not with weakly pathogenic, isolates of the fungus (136). Susceptibility to R. solani and Thielaviopsis basicola was greatly enhanced in cotton seedlings infected by M. incognita (133). Post-emergence damping off of cotton caused by R. solani, Pythium debaryanum or F. oxysporum

f. vasinfectum was remarkably increased in the presence of M. incognita acrita (89).

Brodie and Cooper (15) found that infection by M. incognita, M. hapla, Rotylenchulus reniformis, or Hoplolaimus tylenchiformis prolonged susceptibility of cotton seedlings to Rhizoctonia solani. Prolonged susceptibility to Pythium debaryanum was obtained only in the presence of M. incognita or M. hapla. They attributed prolonged susceptibility to Rhizoctonia to reduction in growth rate of the host due to the nematodes. Prolonged susceptibility to P. debaryanum was associated with a physiological response to the nematode infection. Sporangial production of the fungus was approximately ten times greater in the presence of juice extracted from galled tissue produced by M. incognita than that produced in the presence of juice extracted from healthy root tissue (15). Sayed (108) reported that Hoplolaimus sp., Tylenchorhynchus sp., or Trichodorus sp. had no effect on the severity of damping off of cucumber, peas, and tomato caused by either R. solani or P. ultimum.

Root rot diseases.--Field surveys showed that Meloidogyne incognita acrita increased incidence of cotton root rot caused by Thielaviopsis basicola (8). Apt and Koike (2, 3) studied relationship of M. incognita acrita and Helicotylenchus nannus to pathogenicity of Pythium graminicola on sugarcane. Helicotylenchus nannus had no influence on severity of the root rot (2). However, infection by M. incognita acrita significantly reduced top growth but not root growth of sugarcane caused by the fungus (3). Aphelenchus avenae remarkably reduced severity of root rot of oats caused by P. arrhenomans (102). The nematode apparently controlled the disease by feeding on the fungus. Wyllie and Taylor (137)

reported that a combination of M. hapla and Phytophthora sojae resulted in more root damage to soybeans than either pathogen alone.

The black-shank root-knot disease complex of tobacco has been studied extensively. Tisdale (127) observed that infection by Heterodera marioni (Meloidogyne sp.) reduced resistance of tobacco to the black shank fungus. Similar findings were reported by Moore et al. (77). Fumigation with nonfungicidal nematocides effectively reduced the severity of black shank of tobacco grown on fields infested with Meloidogyne sp. and Phytophthora parasitica var. nicotianae (91). Sasser et al. (107) demonstrated that M. incognita acrita and M. hapla greatly increased severity and rate of development of the fungal disease in black shank-resistant tobacco varieties. Since the fungus is capable of direct penetration of epidermal cells (90) Sasser et al. concluded that the nematodes somehow altered the physiology of host resistance to Phytophthora. Graham (37) showed that M. incognita acrita significantly increased root rot severity in the black shank-resistant tobacco variety Dixie Bright 101. In contrast, Pratylenchus brachyurus, Rotylenchus brachyurus and Tylenchorhynchus claytoni contributed very little, if any, to black shank development. Powell and Nusbaum (97) reported that severity of black shank in root-knot susceptible segregates of tobacco line was significantly greater than that in root-knot resistant segregates of the same line. Incorporation of root-knot resistance also contributed to the black shank resistance. Histopathology of the interaction between either M. incognita or M. incognita acrita and P. parasitica var. nicotianae has been critically investigated (98). Wounding by the nematodes contributed very little to the fungus development. Hyperplastic

and hypertrophic tissues of the nematode galls were extensively colonized by the fungus. Mycelium was often observed within the gelatinous matrixes of the nematode egg masses. Bird (12) reported that egg sacs of M. javanica and M. hapla are largely composed of a glucoproteinaceous substance. The protoplasm of giant cells was highly sensitive to the fungal invasion, whereas a congenial relationship existed between the host and the fungus in the hyperplastic region of the nematode-galled tissue. Powell and Nusbaum (98) concluded that in black shank-susceptible and resistant tobacco varieties, infection by the root-knot nematode evidently altered the physiology of the host, offering the fungus a better substrate for extensive colonization of tissues surrounding nematode infection loci.

Good (36) reported the constant association of Pratylenchus brachyurus with Sclerotium rolfsii on peanuts, but the nature of the association was not determined. Jenkins (50, 51, 52) studied the relation of Thielaviopsis basicola, Fusarium sp., Pythium sp., and Pratylenchus penetrans to brown rot of tobacco. He concluded that neither this nematode nor any of the fungi can produce the disease syndrome. Mountain (79), however, demonstrated that the nematode is the primary cause of the brown rot. Fungi were involved only in later stages of tissue disintegration. Miller (74) found that combined fungicidal and nematocidal chemicals gave better control of strawberry black root rot than did either type of chemical alone. Pratylenchus penetrans and several soil-borne fungi are frequently associated with the disease complex. Reduction in populations of the nematode by soil fumigation did not result in any significant decrease in severity of the black root rot (99).

The etiological role of the nematode or any of the associated fungi has not been elucidated. Mountain and Benedict (80) reported the constant association between P. minyus and Rhizoctonia solani on wheat. The presence of both the nematode and the fungus is essential for development of root rot of winter wheat (9). Norton (88) noted that P. hexincisus increased severity of charcoal root rot of sorghum caused by Macrophomina phaseoli. Similarly, P. pratensis increased severity of tomato root rot caused by Cylindrocarpon radiculicola (41). Triffitt (128) recorded a frequent association of Heterodera schachtii (H. rostochiensis) with Colletotrichum atromentarium; the cause of black dot root rot of potato. Tylenchorhynchus martini interacted synergistically with virulent, but not with weakly pathogenic, isolates of Aphanomyces euteiches; causing common root rot of canning peas (39). The synergistic action was directly related to levels of the nematode inocula. Synergism occurred even at levels of fungal inoculum that was nonpathogenic in an absence of the nematode. It was concluded that the nematode apparently altered the physiology of host resistance to the fungus. Epstein and Griffin (25) reported an association of Xiphinema americanum and Cytospora kunzei var. picae on spruce. They believed that the nematode presumably provided wounds for the fungal invasion. Anguina tritici is reported to be an essential inoculating agent for Dilophospora aleropecuri; the cause of leaf spot and head blight of wheat (5, 59).

Vascular wilt diseases.--McGuire et al. (71) tested the effects of root-knot nematodes Meloidogyne hapla, M. javanica, M. incognita, M. incognita acrita and M. arenaria on Fusarium wilt of alfalfa caused by Fusarium oxysporum f. vasinfectum. Each of the nematode species except

M. incognita acrita significantly increased wilt incidence in the wilt-susceptible variety Buffalo. Wilt severity was positively correlated with root-knot severity. Giamalva et al. (34) found that none of the above listed root-knot nematode species influenced development of Fusarium wilt (F. oxysporum f. batatas) in wilt-susceptible and wilt-resistant sweet potato varieties. The root-knot nematode-Fusarium complex of cotton has been studied extensively. Atkinson (6) pointed out the importance of root-knot nematodes in reducing wilt resistance of various cotton varieties. Smith (114, 115, 116, 119) found that Heterodera marioni (Meloidogyne sp.) greatly increased susceptibility of cotton to Fusarium wilt. Soil fumigation with nematocides markedly controlled the Fusarium-nematode complex. Similar results were also obtained by Taylor et al. (123) and by Jones (54). The Fusarium wilt pathogen is somehow aided by the root-knot nematode in penetration (117). Root-knot resistance and Fusarium wilt resistance were positively correlated in cotton (117, 118, 120). Smith (116, 117) emphasized the role of the root-knot nematodes as predisposing factors to the Fusarium wilt, and stated that breeding for wilt and nematode resistance must be incorporated.

Soil fumigation with ethylene dibromide effectively reduced nematode populations and controlled Fusarium wilt in the wilt-resistant cotton variety Coker 100, grown in fields infested with Meloidogyne incognita, M. incognita acrita, Trichodorus sp., Tylenchorhynchus sp., and Helicotylenchus sp. (87). Martin et al. (57, 68) showed that M. incognita and M. incognita acrita significantly increased wilt severity caused by Fusarium oxysporum f. vasinfectum in wilt susceptible and wilt-

resistant cotton varieties. Different isolates of the root-knot nematodes varied in their ability to predispose the wilt-resistant variety Coker 100 to Fusarium wilt. Species of Trichodorus, Tylenchorhynchus and Helicotylenchus, although reproduced abundantly on cotton, failed to affect wilt severity. In contrast, the stunt nematode (T. claytoni) greatly increased incidence of Fusarium wilt (F. oxysporum f. nicotianae) in the wilt-susceptible tobacco variety Oxford 1-181 (43). Hollis (47) demonstrated a positive correlation between extent of vascular discoloration of cotton caused by Fusarium and infection by M. incognita acrita. He showed that cotton varieties apparently possessed equivalent resistance or susceptibility to the Fusarium-root-knot complex. Similarly, the nematode significantly reduced resistance to Fusarium wilt in the wilt-tolerant and moderately nematode-resistant cotton var. Auburn 56 (33). Fusarium alone did not produce wilting, and produced only 10 per cent vascular discoloration. Similar results were obtained using Deltapine-15, a root-knot and wilt-susceptible variety; and AXTE-1, an experimental breeding line.

Cooper and Brodie (22, 23) studied the role of Meloidogyne incognita acrita and Belonolaimus longicaudatus in predisposing cotton varieties to Fusarium wilt. A highly significant positive correlation between infection by either of these nematodes and Fusarium wilt indices was found. Both nematodes similarly predisposed resistant and wilt-susceptible varieties to fungal infection. Varieties in which root-knot resistance contributed to wilt resistance were also resistant to the sting nematode-Fusarium complex. Similar findings showed that the sting nematode broke down resistance of the cotton variety Coker 100

to Fusarium wilt and significantly increased wilt severity in wilt-susceptible cotton varieties (44, 45, 46). Infection by a reniform nematode (Rotylenchulus reniformis) markedly increased incidence of Fusarium wilt in wilt-susceptible but not wilt-resistant cotton varieties (85). Minton and Minton (76) studied the histopathology of the interaction between M. incognita acrita and Fusarium oxysporum f. vasinfectum on cotton. They showed that the fungus grew profusely in the xylem element and in giant cells. Infection by the nematode apparently provided a suitable substrate for fungal growth. It was also noted that the only portals for entry of the fungus to xylem vessels were through the nematode-decayed tissue.

Soil fumigation with ethylene dibromide was more effective in reducing severity of Fusarium wilt in wilt-resistant than in wilt-susceptible cowpea varieties (125, 126). The authors found that Meloidogyne javanica broke down resistance of the Grant variety to Fusarium wilt and significantly increased wilt severity in the wilt-susceptible variety Chino 3. Inoculation with Fusarium oxysporum f. tracheiphylum alone gave no wilt in the resistant variety and only traces in the susceptible variety. Inoculation by dipping transplants in Fusarium spore suspension, however, resulted in slight xylem necrosis in the wilt-resistant variety compared to severe xylem necrosis of the susceptible variety. They concluded that the role of the nematode is apparently physiological rather than that of merely providing wounds for fungal penetration. Similarly, M. incognita acrita and M. hapla broke down resistance of the pea variety Pluperfect to F. oxysporum f. pisi Race 1, and increased rate of wilting in the wilt-susceptible variety Alaska (24).

Tylenchorhynchus claytoni had no influence on Fusarium wilt in both pea varieties. In addition, histopathological studies revealed the presence of the fungus in the nematode-galled tissue of the susceptible variety. This was accompanied by gum-like deposits, coarse granulation, and disintegration of giant cell contents. Fungus mycelium was also present in giant cells of the resistant variety, but disintegration of cell contents was not evident. Labruyere et al. (52) reported that Hoplolaimus uniformis significantly increased severity of pea wilt caused by F. oxysporum f. pisii Race 3.

Meloidogyne incognita acrita and M. javanica greatly enhanced the incidence of mimosa wilt caused by Fusarium oxysporum f. pernicosum (35). M. incognita acrita, M. incognita, M. hapla, M. arenaria, M. arenaria thameisi, and M. javanica, interacted synergistically with F. oxysporum f. dianthi on carnation (110, 111), whereas Ditylenchus sp. markedly inhibited the development of Fusarium wilt (109). Helicotylenchus nannus and H. buxophilus, although pathogenic on carnation, did not interact with Fusarium (111). Artificial wounding greatly increased carnation wilt. It was concluded that the root-knot nematodes, being endoparasitic were more effective in providing direct pathways to the vascular system for the wilt pathogen than the ectoparasitic nematodes. Soil fumigation with nematocides greatly reduced Fusarium wilt severity of tobacco grown on root-knot infested soils (78). Powell (96) stated that Fusarium wilt of tobacco was more severe when the fungus was added two to four weeks following root-knot nematode infection, than when nematodes and fungus were added simultaneously. Newhall (36) found that the presence of Radopholus similis significantly increased wilt

severity in the banana variety Gros Michel infected by F. oxysporum f. cubense, whereas Meloidogyne sp. had no influence on the severity of wilt. Loos (62) reported that neither R. similis nor M. incognita acrita is prerequisite for Fusarium infection when fungus spores were used at 14 millions/sq. in. of soil. High inoculum level of R. similis, however, shortened the time interval prior to wilt appearance. The role of the burrowing nematode was attributed to providing wounds for fungus penetration (36).

The root-knot-nematode-Fusarium interaction on tomato has long been recognized. Young (138) noted that resistance of tomato to Fusarium wilt was greatly reduced following plantings of root-knot-susceptible varieties. Fumigation with ethylene dibromide did not control the Fusarium-root-knot complex (40), whereas chloropicrin was effective (139). McClellan and Christie (69), however, found that Heterodera marioni (Meloidogyne sp.) had no influence on wilt development. Jenkins and Coursen (53) studied the interaction between M. incognita acrita and M. hapla with Fusarium oxysporum f. lycopersici in tomato. These nematodes had no effect on the wilt-susceptible variety Redbeefsteak, but significantly increased wilt severity in the wilt-tolerant variety Rutgers and broke down resistance of the highly wilt-resistant variety Chesapeake. They concluded that the nematodes evidently altered the physiology of host resistance to Fusarium. In contrast, Binder and Hutchinson (11) found that M. incognita acrita did not alter resistance of the Chesapeake variety to Fusarium wilt, either under normal or potassium deficient conditions. They concluded that differences in inoculum potential and possibly parasitic races of the nematode might account for

the failure to reproduce the previous findings. "Parasitic" races of the root-knot nematode have been reported (66).

Severity of Fusarium wilt of cotton was constantly associated with distribution of populations of the meadow nematode (Pratylenchus pratensis) (113).

The Verticillium-root lesion nematode complex of eggplant was studied extensively (72, 81, 82). Pratylenchus penetrans interacted synergistically with Verticillium albo-atrum at both low and intermediate levels of the fungal inoculum (72, 81). Also, reproduction of the nematode was greatly increased in the presence of the fungus. Nematode populations were decreased, however, by increasing the fungal inoculum. Similarly, the presence of Verticillium increased the final population of the root lesion nematode in tomato, pepper and eggplant (82). The authors suggested that this relationship apparently represents a different type of interaction whereby the nematode and the fungus mutually benefit each other in colonizing the host tissue. Soil fumigation with "Trizone" or "Picfume" effectively reduced populations of P. minyus, P. hamatus, and P. capitatus, and controlled Verticillium wilt (V. albo-atrum f. menthae) of peppermint (27). In contrast, Dichloropropane-dichloropropane reduced only the initial incidence of the Verticillium wilt. Bergeson (10) found that differences in growth reduction of peppermint caused by P. penetrans and V. albo-atrum, alone or in combination, were either nonsignificant or additive. Simultaneous inoculation with the nematode and the fungus had no influence on severity of the Verticillium wilt. However, addition of Verticillium two months after inoculation with the nematode significantly increased

rate of wilting.

Various antifungal treatments, particularly Pentachloronitrobenzene (PCNP), stimulated reproduction of Pratylenchus penetrans and consequently increased the incidence of Verticillium wilt of strawberry (103). The nematode significantly increased the severity and rate of the Verticillium wilt on the wilt-susceptible strawberry variety Dixieland (1). The meadow nematode, although pathogenic, did not interact with Verticillium albo-atrum in moderately and highly wilt-resistant strawberry varieties. Soil coverings of paper or addition of white pine sawdust reduced populations of P. penetrans and controlled Verticillium wilt of tomato (75). These soil amendments alone had no effect on severity of the Verticillium wilt. Mountain and McKeen (83) reported that the meadow nematode greatly increased the severity of tomato wilt caused by V. dahliae.

Soil surveys showed that Meloidogyne incognita acrita was constantly associated with high incidence of Verticillium wilt of cotton (8). Soil fumigation with ethylene dibromide, although reducing populations of the root-knot nematode, did not decrease the incidence of the Verticillium wilt (70). The nature of the interactions between the nematode and V. albo-atrum had not been elucidated.

Bacteria-nematode interactions

Interaction between nematodes and bacteria has received relatively little attention (94). Ark and Thomas (4) reported that infection of apple roots by Anguillulina pratensis (Pratylenchus sp.) greatly enhanced tissue colonization by Pseudomonas fluorescens. The bacterium

alone, however, was nonpathogenic. Ditylenchus dipsaci was reported to be an essential inoculating agent and possibly a vector for development of the crown rot of rhubarb caused by Bacterium rhaponticum (Erwinia rhaponticum) (73). The interaction between Aphelenchoides ritzemabosi and Corynebacterium fascians on strawberry has been thoroughly investigated (95). The cauliflower disease complex consists of two distinct, but intimately related bacterial and nematode diseases. The nematode alone inhibits growth of the apical meristem producing short-lived alamate leaves. Bacteria alone stimulate growth of the dormant meristems producing leafy gall symptoms. Both pathogens are complementary etiological factors for full expression of the cauliflower disease syndrome. The nematode also acts as a vector for the bacterium. Similarly, Anguina tritici apparently acts as a vector for Corynebacterium tritici; the cause of the yellow slime disease of wheat (17, 106, 130). Chaudhuri (17) reported that the bacterium alone failed to infect wheat, whereas Cheo (18) claimed full proof of the bacterial pathogenicity.

Meloidogyne incognita significantly increased the incidence of bean wilt caused by Corynebacterium flaccumfaciens var. aurantiacum, whereas M. hapla had no influence on the wilt development (112). Mechanical wounding greatly increased severity and rate of the bacterial wilt. The role of the nematode was attributed to providing wounds for bacterial invasion. Stewart and Schindler (122) demonstrated that populations of M. hapla, M. javanica, M. incognita, M. incognita acrita, M. arenaria or Helicotylenchus nannus significantly increased rate and severity of carnation wilt caused by Pseudomonas caryophylli. Xiphinema

diversicaudatum had no effect on the wilt development, whereas Ditylenchus sp. markedly decreased severity and rate of wilting. Mechanical wounding also increased rate and severity of wilt. Similarly, M. incognita interacted synergistically with P. solanacearum on tobacco (64). The nematode significantly increased severity and rate of wilting in the moderately wilt-resistant variety Dixie Bright 101 (65). The development of wilt in plants inoculated with the nematode and the bacteria was comparable to that produced in plants inoculated by pouring bacterial suspension on freshly-cut roots. Inoculation with the bacteria 24 or 48 hours after artificial wounding gave significantly lower wilt indices than that produced by simultaneous inoculation with the nematode and the bacteria. Plants inoculated with a concentrated bacterial suspension developed more rapid and more severe wilting than did plants inoculated with one-tenth as many bacteria. In the presence of the nematode, however, wilt severity was greatly increased at both high and low levels of the bacterial inoculum. The authors concluded that the nematode apparently provided suitable wounds for the bacterial invasion.

Tylenchorhynchus claytoni failed to increase susceptibility of wilt-tolerant tobacco varieties to Xanthomonas solanacearum (63). Failure of the stunt nematode to interact synergistically with the bacteria was interpreted on the basis that wounds inflicted by the nematode feeding are too superficial to offer the vascular pathogen a direct pathway to the xylem vessels. According to Kelman (55) Hunger demonstrated that tomato plants were readily infected with Pseudomonas solanacearum when grown in root-knot infested soil. Libman and Leach

(60) reported that fumigation with Dibromochloropropane significantly decreased severity of bacterial wilt of tomato grown on soil infested with P. solanacearum and an unidentified population of plant parasitic nematodes. In addition greenhouse experiments showed that Helicotylenchus nannus markedly increased severity of the bacterial wilt. Similarly, M. hapla and H. nannus significantly increased incidence and severity of the bacterial wilt (61). Artificial wounding of tomato roots was more effective in increasing the severity of wilt than either the root-knot or the spiral nematode.

OBJECTIVES OF THE PRESENT RESEARCH

Two hypotheses have been advanced to account for the role of plant parasitic nematodes in predisposing host plants to fungal and bacterial pathogens. One holds that wounds, inflicted by nematodes through their feeding, offer suitable infection courts that facilitate bacterial and fungal invasion. This is largely based on the findings that nematodes gave significant increases in fungal and bacterial disease severity comparable to that produced by artificial wounding (25, 62, 63, 64, 86, 110, 111, 112, 122). However, exact correlation between nematode invasion and ingress by fungal or bacterial pathogens is lacking.

The second hypothesis states that nematodes apparently alter the physiology of host resistance, thus predisposing them to other pathogens. There are several lines of evidence supporting this hypothesis. Interaction between nematodes and other pathogens is sometimes highly specific in nature (39, 136). In other cases infection by nematodes completely broke down the high resistance of certain host varieties to fun-

gal pathogens (24, 76, 98).

Southern bacterial wilt of tomato caused by Pseudomonas solanacearum is one of the most destructive plant diseases. The exact mechanism of bacterial ingress is not known. In the absence of external root injury, there is evidence suggesting bacterial entry through wounds arising at points of emergence of secondary roots (16). Bacteria readily entered roots of both susceptible and wilt-resistant tomato and tobacco varieties (135). However, wilt susceptibility was significantly correlated with an increase in bacterial population within host tissue. It was suggested that establishment of the pathogen is largely determined by its ability to maintain rapid multiplication and successful parasitic relations with its host. The facts that nematodes having different modes of feeding similarly predispose tomato to the bacteria, that mechanical wounding was more effective than nematodes as predisposing agents (61), and that wilt susceptibility is correlated with successful establishment of parasitic relation (16, 135), suggest that other factors besides woundings might be involved.

The objectives of this investigation were to 1) test the existence of interactions between Meloidogyne incognita acrita and Pseudomonas solanacearum in susceptible and wilt-resistant tomato varieties, 2) study the comparable effect of the nematode and artificial wounding on severity and rate of wilting on both host varieties, and 3) test the hypothesis that, although wounds inflicted by the nematode might be a contributing factor, nematode infection somehow alters physiology of the host in such a way that its susceptibility to the bacterial wilt is increased.

PART I

WILT INCIDENCE IN INTACT NEMATODE-INFECTED AND NEMATODE-FREE PLANTS

Inoculation experiments were conducted in the greenhouse to test the interaction of the nematode Meloidogyne incognita acrita Chitwood 1949, and the bacterium Pseudomonas solanacearum E. F. Sm. on wilt-susceptible and resistant tomato varieties. Experiments also included a study of the comparable effects of the root-knot nematode and artificial wounding on severity and rate of the bacterial wilt of both tomato varieties.

MATERIALS AND METHODS

The virulent isolate 60-Re¹ of Pseudomonas solanacearum was used throughout the inoculation tests. Inoculum was maintained by successive subculturing on potato-dextrose agar (PDA) slants in test tubes. Stock cultures of the bacterium were stored by using sterile mineral oil at 5-10° C as described by Kelman and Jensen (56). Cultures kept under these conditions remained highly pathogenic to the tomato variety Rutgers for two years. The root-knot nematode was originally isolated from a naturally infected Viburnum plant growing in Gainesville, Florida. By using routine methods, perineal patterns of several adult female nematodes were prepared and examined under the

¹Kindly supplied by Dr. Arthur Kelman, Department of Plant Pathology, North Carolina State College, Raleigh, N. C.

compound microscope. The nematode was identified² as Meloidogyne incognita acrita Chitwood 1949.

Several egg masses of the nematode were surface-sterilized for 30 minutes in a solution containing 0.1 per cent CuSO_4 and 1 per cent streptomycin sulfate. Egg masses were then rinsed three times each for ten minutes in distilled water. Tomato seedlings of the Rutgers variety were grown in steam-sterilized potting soil. Plants were inoculated with the nematode by pouring several egg masses suspended in distilled water into a cavity formed in the soil near the plant roots. The cavity was then filled with soil. The nematode inoculum that developed was maintained for two years by repeated planting and transplanting of Rutgers tomato in nematode-infested and initially steam-sterilized potting soil.

Rutgers tomato (Lycopersicon esculentum Mill.) and the tomato breeding line designated as 1961-57-55 MO.P³ were used as host plants in the inoculation tests.

Methyl bromide-treated field soil mixed with peat was steam-sterilized by autoclaving for one hour at 18 psi. Standard 3-inch clay pots were similarly autoclaved and then filled with the sterilized soil. "Arasan"-treated seeds of the tomato variety Rutgers and the tomato line "MO.P" were planted in the potting soil. Several seeds per pot of each host plant were used. Two weeks after planting the tomato

²By Dr. V. G. Perry, Department of Entomology, University of Florida, Gainesville, Florida.

³Seeds kindly supplied by Dr. W. R. Henderson, Department of Horticultural Sciences, North Carolina State College, Raleigh, N. C.

seedlings were thinned. Plants were grown in the greenhouse and watered as frequently as needed.

The tomato variety Rutgers is highly susceptible to bacterial wilt and the tomato breeding line "MO.P" is moderately wilt-resistant.⁴ A standardized inoculating procedure that would permit evaluation of the severity of bacterial wilt in the presence or absence of the root-knot nematode was developed. Several bacterial subcultures were grown on PDA slants in test tubes at 28-30° C for three days. Bacterial growth from a three-day old culture was transferred with a wire loop to several 250-ml Erlenmeyer flasks each of which contained 100 ml of sterile potato dextrose broth. Liquid bacterial cultures were shaken for three days at 21° C on a reciprocating shaker. Uninoculated medium was shaken in the same manner. The resultant bacterial growth in the shake liquid culture was used as a standard bacterial inoculum throughout the inoculation tests.

Nematode inoculum was obtained from heavily infected roots of the Rutgers tomato. Plants were carefully removed from the soil and thoroughly washed with tap water. Individual rootlets were examined under the dissecting microscope. Five mature egg masses of approximately the same size were surface-sterilized and were added to each pot of soil as described earlier.

Inoculation of tomato seedlings with the bacterium was done immediately following addition of the nematode to the soil. A 20-ml portion of the bacterial suspension was poured on the surface of the soil

⁴Dr. W. R. Henderson, Department of Horticultural Sciences, North Carolina State College, Raleigh, N. C. Personal communication.

around the plant roots in each pot. Equal volumes of sterile media were similarly added to each pot of soil not infested with egg masses of the nematode.

Roots were artificially wounded prior to inoculation with the bacterium by forcing a pair of scissors, previously dipped in 95 per cent ethanol, into the soil and cutting the roots several times. Age of plants at the time of inoculation with the nematode and the bacterium ranged from 28 to 39 days. Following inoculation, plants were thoroughly watered and placed on benches in the greenhouse.

All experiments were designed as randomized blocks with varying number of treatments. Five to eight of the following standard treatments were applied as follows: 1) uninoculated control, 2) sterile potato dextrose broth added, 3) roots wounded only, 4) inoculated with the nematode only, 5) inoculated with the bacterium only, 6) inoculated with the bacterium and roots wounded, 7) inoculated with the bacterium and the nematode, 8) inoculated with the bacterium and the nematode and roots wounded. Treatments were replicated three to five times with five to eight plants per replicate.

Bacterial wilt readings were recorded daily following inoculations until the maximum expressions of wilt in presence or absence of the nematode were reached. Duration of each experiment from the time of inoculation until the final readings ranged from four to five weeks. Wilting, leaf epinasty, vascular discoloration, and presence of bacterial ooze in the infected stems were considered in detecting infection by the bacterium. Bacterial wilt data were calculated as total

percentage of wilted plants in each treatment. Statistical differences among treatments were obtained using the Chi square factorial analysis.⁵ All experiments were conducted in the greenhouse under daily temperature ranges of 25° to 28° C.

RESULTS AND DISCUSSION

In the presence of the nematode, root wounding or both, symptoms of bacterial wilt began to appear much earlier in both host plants than those in plants treated with the bacterium alone. Inoculation with the bacterium and the nematode in the presence or absence of artificial root woundings resulted in more severe stunting of seedling growth than did inoculation with the bacterium alone. Uninoculated controls, plants treated with sterile PDB, or root wounding remained healthy. Occasional wilting of plants inoculated with the nematode alone appeared especially at later stages of the root-knot infection. However, there was no evidence of bacterial infection in those plants due to contamination.

Infection by the root-knot nematode markedly increased the incidence of bacterial wilt in the wilt-susceptible tomato variety Rutgers and the wilt-resistant "MO.P" tomato breeding line. The incidence of bacterial wilt in plants inoculated with the bacterium and the nematode was significantly higher than that in plants inoculated with the bacterium alone (Tables 1, 3, 4). Similarly, wounding significantly increased the incidence of bacterial wilt in both host plants (Tables

⁵Dr. E. Brandt, former Statistician Head, Agr. Expt. Sta., University of Florida, Gainesville, Fla. Personal communication.

2, 3, 4). With one exception, the increased incidence of wilt in presence of the nematode or wounding was statistically significant. These results strongly support the earlier findings reported by Libman et al. (61).

Data are also similar to those obtained with the Granville wilt of tobacco (65), with Pseudomonas caryophylli in carnations (122) and with Corynebacterium flaccumfaciens var. aurantiacum in beans (112). In addition, the results show that inoculation of freshly wounded roots of both host plants with the bacterium and the nematode gave significantly higher incidence of bacterial wilt than did inoculation with the bacterium alone (Tables 2, 3, 4).

In one experiment using the tomato variety Rutgers the nematode failed to produce any significant increase in the incidence of bacterial wilt. There were no significant differences in the incidence of bacterial wilt between plants inoculated with both pathogens and those inoculated with the bacterium alone (Table 2). Furthermore, artificial root wounding was more effective in increasing susceptibility of the variety Rutgers to the bacterial wilt than the nematode. The combination of the bacterium and wounding resulted in significantly higher incidence of bacterial wilt than did combination of the bacterium and nematode (Table 2). Similar results were reported by Libman et al. (61).

Examination of roots of the variety Rutgers inoculated with the nematode alone revealed only slight galling of the roots (Table 2), compared to the severe root-knot infection obtained in another exper-

TABLE 1. Incidence of Southern bacterial wilt in nematode-free and nematode-infected tomato seedlings^a of the variety Rutgers and the "MO.P" tomato breeding line

Treatment ^b	Total Per Cent Wilt	
	Variety	
	Rutgers	"MO.P"
Uninoculated control	0	0
Sterile potato dextrose broth	0	0
Nematode ^c	0	0
Bacterium	40.0 ^d	35.0 ^d
Bacterium + nematode	80.0	75.0

^aSeedlings were 28 days old at the time of inoculation.

^bAll treatments were replicated 5 times with 8 plants per replicate.

^cSeedlings of both host plants inoculated with the nematode alone showed severe galling of the roots.

^dDifferences between treatments within the same host plant were statistically significant at the 1 per cent level. Differences between comparable treatments of the two host plants were not statistically significant.

TABLE 2. Comparable effects of artificial wounding and infection by the nematode on the incidence of Southern bacterial wilt in the tomato variety Rutgers^a

Treatment ^b	Total per cent wilt
Uninoculated control	0
Sterile potato dextrose broth	0
Roots wounded	0
Nematode ^c	0
Bacterium	36.0
Bacterium + roots wounded	88.0 ^d
Bacterium + nematode	52.0 ^e
Bacterium + nematode + roots wounded	88.0 ^d

^aPlants were 31 days at the time of inoculation.

^bAll treatments were replicated 5 times with 5 plants per replicate.

^cPlants inoculated with the nematode alone, gave an average of 82 galls per gm of fresh root tissue per replicate.

^dDifferences from treatment with bacterium alone were statistically significant at the 1 per cent level.

^eDifferences from treatment with bacterium alone were not statistically significant. Differences from treatment with bacterium and wound were statistically significant at the 1 per cent level.

TABLE 3. Comparable effects of artificial wounding and infection by the nematode on the incidence of Southern bacterial wilt in the "MO.P" tomato breeding line^a

Treatment ^b	Total per cent wilt
Uninoculated control	0
Sterile potato dextrose broth	0
Roots wounded	0
Nematode ^c	0
Bacterium	13.33 ^d
Bacterium + roots wounded	46.66 ^e
Bacterium + nematode	93.33
Bacterium + nematode + roots wounded	86.66

^aPlants were 28 days old at the time of inoculation.

^bAll treatments were replicated 3 times with 5 plants per replicate.

^cPlants inoculated with the nematode alone showed severe galling of the roots.

^dDifferences from all other treatments were statistically significant at the 1 per cent level.

^eDifferences from the treatment bacterium + nematode were statistically significant at the 1 per cent level.

TABLE 4. Comparable effects of artificial wounding and infection by the nematode on the incidence of Southern bacterial wilt in tomato seedlings^a of the variety Rutgers and the "MO.P" breeding line

Treatment ^b	Total Per Cent Wilt	
	Variety	
	Rutgers	"MO.P"
Uninoculated control	0	0
Sterile potato dextrose broth	0	0
Roots wounded	0	0
Nematode ^c	0	0
Bacterium	36.0 ^d	4.0 ^d
Bacterium + roots wounded	96.0	52.0
Bacterium + nematode	88.0	40.0
Bacterium + nematode + roots wounded	100.0	64.00

^aPlants were 39 days old at the time of inoculation.

^bAll treatments were replicated 5 times with 5 plants per replicate.

^cPlants inoculated with nematode alone gave an average of 132 and 136 galls per gm of fresh root tissue per replicate of Rutgers and "MO.P" line respectively.

^dDifferences from all other treatments of the same host plant were statistically significant at the 1 per cent level. Differences between comparable treatments of the two host plants were statistically significant at the 1 per cent level.

iment (Table 4). It was generally observed that the severity of root-knot infection was associated with significant increase in the incidence of bacterial wilt in both host plants. Schindler and Stewart (111) reported similar observations with the root-knot-Fusarium interaction on carnation. Although the inoculum level of the nematode used in these tests was not rigidly controlled, a decrease in the nematode population due to unfavorable environmental conditions may account for failure of the nematode to produce significant increase in the incidence of bacterial wilt.

In cases where root-knot infection was severe, the increase in the incidence of bacterial wilt produced in presence of the nematode was either comparable to (Table 4) or significantly higher than that produced by artificial wounding (Table 3). The increase in wilt incidence produced in presence of the nematode and wounding was not significantly different from that produced in presence of the nematode or wounding (Table 4). In one experiment using the "MO.P" line, treatment with artificial root wounding, the nematode and the bacterium gave significantly higher incidence of bacterial wilt than did treatment with the bacterium and wounding (Table 3).

Differences in susceptibility of the tomato variety Rutgers and the tomato "MO.P" line to the bacterial wilt in the presence or absence of the nematode were not statistically significant (Table 1). When age of the plants at the time of inoculation was increased from 28 to 39 days susceptibility of the "MO.P" tomato line to bacterial wilt was remarkably decreased. Differences in the incidence of bacterial wilt be-

tween the two host plants treated with the bacterium alone, the bacterium and the nematode, the bacterium and root wounding, or the combination of bacterium, nematode and wounding were highly significant (Table 4). The decreased susceptibility of the "MO.P" line to the bacterial wilt is presumably due to changes in the host tissues associated with the increased age of seedlings. These results support the previous findings reported by Winstead and Kelman (1935).

Data also show that artificial root wounding, infection by the nematode or their combined effects significantly increased the rate of bacterial wilt development in both host plants (Table 5). In all cases artificial root wounding was more effective in increasing the rate of development of bacterial wilt than the nematode. The increased rate of bacterial wilt in the presence of artificial root wounding was significantly higher than that produced in the presence of the nematode (Table 5).

These differences could be explained on the basis that artificial root wounding is most likely to offer an extensive suitable infection courts for massive bacterial invasion within relatively short period of time prior to wound healing. Inoculum of the nematode used in the inoculation tests was introduced as egg masses. Hatching of the eggs and release of the infective larvae was presumably gradual. Nematode larvae apparently invaded plant roots over a prolonged period of time. This, in part, may account for the more rapid increase in the rate of bacterial wilt caused by artificial wounding compared to that produced by the nematode. It is also possible that the actions

TABLE 5. Effects of artificial wounding and infection by the nematode on the rate of development of Southern bacterial wilt in tomato seedlings of the variety Rutgers and the "MO.P" breeding line

Experiment ^a	Variety of line	Rate ^b of wilting Treatment ^c			
		B	B+N	B+W	B+N+W
1	Rutgers	1.81 ^d	3.63		
1	"MO.P"	1.75 ^d	4.16		
2	Rutgers	2.40 ^e	2.36	5.86 ^f	8.00
3	"MO.P"	0.95 ^d	4.44	6.66 ^g	8.66
4	Rutgers	1.71 ^d	4.00	6.85 ^f	7.14
4	"MO.P"	0.26 ^d	1.66	3.71 ^f	3.55

^aExperiment number 1, 2, 3 and 4 refers to data represented in tables 1, 2, 3 and 4, respectively.

^bRate of wilting is expressed as mean percentage of daily wilt. Values obtained by dividing total percentage of bacterial wilt by number of days starting from time of inoculation until maximum expression of the wilt in a given treatment was reached.

^cSymbols indicate respective treatments:

B = Inoculated with bacterium.

B+N = Inoculated with bacterium + nematode.

B+W = Inoculated with bacterium + root wounded.

B+N+W = Inoculated with bacterium + nematode + roots wounded.

^dDifferences from the treatments B+N, B+W, and B+N+W were statistically significant at the 1 per cent level.

^eDifference from the treatment B+N was not statistically significant.

^fDifferences from the treatment B+N were statistically significant at the 1 per cent level.

^gDifference from the treatment B+N was statistically significant at the 5 per cent level.

of the nematode and artificial wounding on host tissue are entirely different in nature.

Results of the present work clearly demonstrate the interaction of the nematode and the bacterium. Similar interaction of the bacterium and artificial root wounding is evident. The role of the root-knot nematode in predisposing both host plants to the bacterial wilt was not determined. Several authors (65, 112, 122) have advanced the hypothesis that wounds inflicted by the nematode during penetration may provide suitable infection courts for massive bacterial invasion. Root-knot larvae penetrate directly through the root epidermis near the root tip destroying some epidermal cells during penetration (19). This undoubtedly produces some pathways through which the bacterium may gain ingress. However, exact correlation of larval penetration and bacterial invasion of host tissue is lacking.

Wounding of various causes are apparently essential for ingress by Pseudomonas solanacearum (55). In an absence of external injury there is evidence suggesting that bacteria enter through wounds that arise at points of emergence of secondary roots (16). The bacterium readily entered roots of susceptible and wilt-resistant tomato varieties (135). Wilt susceptibility was positively correlated with extensive tissue colonization by the bacterium and establishment of a successful parasitic relationship with the host (16, 135). Results of the present investigation indicated that the nematode and artificial wounding might not be equally effective in predisposing tomato varieties to the bacterial wilt. In addition artificial root wound-

ing was more effective in increasing the rate of bacterial wilt than the nematode. This may suggest that the mode of action of the two predisposing agents might be different. It is, therefore, suggested that although wounds inflicted by the nematode may, in part, account for the increased susceptibility of both host plants to the bacterial wilt, other factors beside mere wounding might be involved.

PART II

WILT SEVERITY OF EXCISED SHOOTS FROM NEMATODE-INFECTED AND NEMATODE-FREE PLANTS TREATED WITH BACTERIA-FREE CULTURE FILTRATES

Results of the inoculation experiments reported earlier clearly demonstrated that infection by the root-knot nematode significantly increased the incidence of bacterial wilt in susceptible and wilt-resistant host plants. Laboratory experiments were conducted to test the hypothesis that the nematode somehow altered the physiology of the host so that its susceptibility to the bacterial wilt was increased.

MATERIALS AND METHODS

The isolate 60-Re of Pseudomonas solanacearum and the nematode Meloidogyne incognita acrita were used throughout this investigation. Maintenance of inocula of both pathogens, methods of soil sterilization, planting, and inoculation with the nematode were carried out in the same manner as described previously. Rutgers tomato and the tomato line designated "1961-57-55. MO.P" were used as host plants. Plants were individually grown in steam-sterilized soil in standard three-inch clay pots. Seedlings were 48 days old at the time of inoculation with the nematode. Plants were maintained in the greenhouse for six to nine weeks after inoculation with the nematode. Plants not inoculated with the nematode were similarly treated.

Five laboratory experiments were conducted to test the effects of culture filtrates of the bacterium on the severity of bacterial wilt

in shoots from healthy and nematode infected plants. Unless otherwise indicated, the methods of culturing the bacterium and preparation of the culture filtrate were accomplished in the same manner in all experiments. Two liquid media were used for preparing the bacteria-free culture filtrates. In experiment 1, the synthetic medium described by Husain and Kelman (46) was used. In experiments 2, 3, 4 and 5 the bacterium was grown in potato-dextrose broth.

Stock cultures of the bacterium were grown on potato dextrose agar slants at 28-30° C for three days. The bacterial growth from each of several three-day old cultures was suspended in ten ml of sterilized distilled water. These ten ml portions of the bacterial suspension were poured into each of several 500-ml Erlenmeyer flasks containing 250-ml sterile medium. In experiments 1, 2, and 3 bacterial liquid cultures were shaken for 48 hours at 70° F on a reciprocating shaker. In experiments 4 and 5 bacterial cultures were shaken at 82-86° F. Uninfested media were similarly treated.

Bacterial shake cultures were centrifuged at 5000 g for one hour. The supernatant fluids were filtered by suction through clarifying pads with a pore diameter of 1 u. The clarified supernatant fluids were sterilized by suction filtration through Seitz filter pads with a pore diameter of 0.1 u. The resulting bacteria-free culture filtrates were tested later for their effect on the tomato excised shoots. Uninfested liquid media were treated in the same manner.

Unless otherwise specified, all experiments to determine induction of bacterial wilt using the culture filtrates were carried out in the following manner. Nematode-infected and healthy seedlings of each

host plant were carefully removed from the potting soil. Plant roots were thoroughly washed with tap water. The basal portion of the stems were severed and cuttings were placed for two to three hours in containers filled with tap water. Only excised shoots with apparent full turgidity were used in the experiments with the culture filtrates.

Fifteen-ml portions of the sterile culture filtrate were poured into 20-ml sterilized test tubes. Sterilized distilled water and sterile media served as controls. Stem cuttings were dipped in 95 per cent ethanol for one minute and rinsed in sterilized distilled water to remove the alcohol. The basal portion of each stem cutting was severed under sterilized distilled water and then immersed individually in the test solutions. In experiments 1, 2 and 3 uniform cuttings with five to seven well-expanded leaves were used. Cuttings in the test solutions were held at 70° F. In experiment 4 and 5 individual cuttings of stems bearing five leaves were placed in the test solutions and held at 76-82° F.

All laboratory experiments were designed as randomized blocks. Stem cuttings from healthy and nematode infected seedlings of both host plants were treated as follows: 1) with sterilized distilled water, 2) with sterile culture medium, 3) with sterile culture filtrates. Treatments were replicated five to six times with one cutting per replicate. Stem cuttings were examined for symptoms of wilting for ten hours at 2.5 hour intervals.

Wilt indices were obtained by using an arbitrary scale for measuring the severity and rate of wilt development in each cutting.

Higher numerical values were assigned to wilting that developed earlier. In experiments 1 and 2 wilt severity was rated as follows: 0, no wilting; 1, partial wilting; 2, complete wilting. Partial wilting that occurred 2.5, 5.0, 7.5, and 10 hours after treatment with the test solutions was assigned the following values: 5, 4, 3 and 2 respectively. Complete wilting that occurred at the above indicated time intervals was assigned the following values: 10, 8, 6 and 4 respectively. In experiments 3, 4, and 5 wilt severity was rated as follows: 0, no wilting; 1, one leaf wilting; 2, two leaves wilting; 3, three leaves wilting; 4, four leaves wilting; 5, five leaves wilting. Wilting that developed 2.5, 5, 7.5, and 10 hours after treatment with the test solutions were assigned the numerical values 10, 9, 8, and 7 respectively. Thus, in all experiments higher wilt indices were obtained when complete wilting of cuttings occurred rapidly. Differences between treatments were measured statistically by the Chi square factorial analysis.

After wilt ratings were obtained, cuttings were held in the test solutions for periods up to 48 hours. At the end of each experiment, the basal portion of each cutting was severed and then cuttings were transferred to sterilized distilled water so that possible recovery of turgor could be noted.

RESULTS AND DISCUSSION

A preliminary experiment to test the effects of infection by the nematode on incidence of the bacterial wilt using bacteria-free culture filtrate was conducted. Three-month old healthy and nematode-infected intact seedlings of Rutgers tomato were treated with the culture filtrate for 15 hours. The results disclosed that incidence of wilt in the nematode-infected plants were four times higher than that in the healthy plants. This suggested that the nematode altered the physiology of the plant, thus predisposing it to the bacterial wilt. Further experiments were carried out to obtain more evidences supporting this hypothesis. In order to rule out the possibility that root wounding inflicted by the nematode might account for the observed increase in wilt incidence, excised shoots from healthy and nematode-infected plants were used.

Results of five laboratory experiments are summarized in Table 6. The excised shoots began to exhibit symptoms of wilting within five hours after treatment with sterile culture filtrates of the bacterium. The results showed that shoots from nematode-infected seedlings of each host plant developed more rapid and more severe wilting than did shoots from plants free of the nematode. Wilting of shoots treated with the culture filtrates is apparently due to toxic metabolite(s) of the bacterium produced in the culture media.

Twelve hours following treatment with the test solutions, occasional wilting of shoots placed in the sterile media began to appear.

TABLE 6. Wilt indices of shoots from nematode-infected and shoots from healthy tomato plants of the variety Rutgers and the bacterial wilt-resistant "MO.P" line ten hours after treatment with sterile culture filtrates of Pseudomonas solanacearum.

Experiment ^a	Variety	Wilt index ^b	Wilt index ratio healthy/nematode- infected
Experiment 1.			
Healthy	Rutgers	3.20 ^c	
Nematode-infected	Rutgers	6.25	0.51
Experiment 2.			
Healthy	Rutgers	2.16 ^d	
Nematode-infected	Rutgers	2.50	0.86
Experiment 3.			
Healthy	Rutgers	5.8 ^c	
Nematode-infected	Rutgers	15.4	0.38
Experiment 4.			
Healthy	Rutgers	2.00 ^c	
Nematode-infected	Rutgers	7.60	0.26
Healthy	"MO.P"	10.20 ^c	
Nematode-infected	"MO.P"	17.60	0.58
Experiment 5.			
Healthy	Rutgers	3.60 ^c	
Nematode-infected	Rutgers	14.80	0.24
Healthy	"MO.P"	2.00 ^c	
Nematode-infected	"MO.P"	5.60	0.36

^aTreatments in experiments 1, 4 and 5 were replicated five times, and those in experiment 2 and 3 were replicated six times.

^bProducts of calculations based on arbitrary numerical values assigned to measure severity and rate of wilt development.

^cWithin a variety; differences from wilt indices of shoots from nematode-infected plants were statistically significant at the 1 per cent level.

^dDifferences between wilt indices of shoots from nematode-infected and healthy plants were not statistically significant.

The sterile media became turbid and turned whitish in color. Microscopic examination revealed that the sterile media were contaminated with a saprophytic bacterium. There was no apparent contamination of the culture filtrate. When these wilted shoots were transferred to sterilized distilled water they recovered completely.

In contrast, wilted shoots treated with the culture filtrate either did not recover or reached only partial recovery.

Data presented in Table 6 demonstrate that, with one exception (experiment 2), treatment with the culture filtrates resulted in significantly higher wilt indices of shoots from nematode-infected seedlings of either host plant than those of shoots from healthy seedlings. In experiment 2 it was observed that nematode infected plants showed only slight galling of the roots compared to the severe galling of the roots obtained with other comparable experiments. It is therefore suggested that a decrease in population of the nematode, due to unfavorable environmental conditions, might in part account for the inability to reproduce these results.

A preliminary experiment was conducted in an attempt to obtain evidence supporting this explanation. Three-month old seedlings of Rutgers tomato infected by the root-knot nematode were used. Severity of root-knot infection was rated on the basis of visual observation of the amount of root galling. Uniform shoots from heavily galled and slightly galled plants were treated with culture filtrates of the bacterium for twelve hours. Shoots from healthy seedlings were similarly treated and served as controls. All treatments were replicated four times with one

shoot per replicate. Wilt severity of the shoots were rated on an arbitrary scale as follows: 0, no wilting; 1, partial wilting; 2, complete wilting. Wilt indices were calculated as mean average of four replicates. The results showed that wilt indices of shoots from heavily galled plants were significantly higher than those of shoots from slightly galled plants. There were no significant differences between wilt indices of shoots from healthy plants and those from slightly galled plants. These results indicate that an increase in severity of root-knot infection intensifies changes in the host tissue that are presumably responsible for the increased wilt severity, and provide the most likely explanation for the anomalous results obtained in experiment 2.

Treatment with the culture filtrates gave significantly higher wilt indices of shoots from nematode-infected plants of the "MO.P" line than those of shoots from nematode-infected plants of the variety Rutgers. Similarly, wilt indices of shoots from healthy plants of the "MO.P" line were significantly higher than those of shoots from healthy plants of the variety Rutgers obtained in experiment 4 (Table 6). Results of inoculation experiments (Table 4) demonstrated that at the age of 39 days, plants of the "MO.P" tomato line showed significantly higher resistance to bacterial wilt than did the variety Rutgers. This significant differences in wilt susceptibility occurred even when plant roots of both host plants were artificially wounded which suggests that resistance of the "MO.P" line is presumably plasmatic in nature. This is supported by the findings that bacteria readily en-

tered roots of both susceptible and wilt-resistant tomato varieties (135). It seems probable that resistance of the "MO.P" line to bacterial wilt is more related to inability of the pathogen to maintain rapid multiplication and to establish a successful parasitic relation with its host, than to detoxification of the toxic metabolites of the bacterium by the host. This is supported by the results that excised shoots from nematode-infected plants and shoots from healthy plants of the wilt resistant "MO.P" line were highly susceptible to toxic metabolites of the bacterium produced in culture.

Results of these investigations strongly demonstrate that infection by the root-knot nematode predisposed excised shoots of susceptible and moderately-wilt resistant host plants to toxic metabolite(s) of the bacterium. This is the first direct evidence supporting the hypothesis that the nematode somehow alters the metabolism of the host so that its susceptibility to the bacterial wilt is increased. The physiological nature of such changes in host metabolism was not determined. Damage to plants by the root-knot nematode may result in mechanical injury during larval penetration, nutritional depletion resulting from withdrawal of cell contents, and biochemical injury due to the enzymatic action of the esophageal secretion (21). Plant-nematode biochemical interactions are considered to be primarily responsible for plant injury; thus mechanical damage is generally insignificant (57).

There are several possible mechanisms by which the root-knot nematode may alter the physiology of the host so that predisposition to

the bacterial wilt occurs. It has been postulated that the nematode directly stimulates bacterial growth by either providing a suitable metabolite or by modifying the host tissue to a better substrate for the development of the bacterium and its pathogenic activities. Enzyme production by the nematode and the biochemical effects of these enzymes on the physiology of the host have been investigated (13, 14, 84, 93). Histochemical studies showed that the hyperplastic regions and giant cells in galls of tomato roots infected by Meloidogyne incognita acrita are the sites of increased metabolic activities (13, 93). Cytoplasm of giant cells contained ten times as much protein as normal cells (13). Owens and Novotny (93) found that protein, nucleic acids, phosphorus and nitrogen increased four times in the nematode-galled tissue. Zinc, magnesium, iron, manganese, sodium, copper and sulfur also increased. It seems logical to assume that these metabolic changes may have some bearings on the nature of the nematode-bacteria interaction. Powell (98) reported that hyperplastic and hypertrophic tissues of tobacco roots infected by M. incognita acrita were extensively colonized by the fungus Phytophthora parasitica var. nicotianae. Similar results were obtained by Minton and Minton (76) with the nematode and Fusarium oxysporum f. vasinfectum in cotton.

Another possible mechanism is that the nematode may, in some manner, predispose the host to bacterial wilt by interfering with the plant nutrition. A decrease in levels of potassium nutrition was positively correlated with a significant increase in severity of the bacterial wilt of tomato (30, 31, 32, 49). Similar correlation was also obtained with

Fusarium wilt of tomato (131). Oteifa (92) reported that severity of infection by Meloidogyne incognita was positively correlated with a depletion of potassium contents in lima beans.

It has been suggested that nematode-infected plants used in the experiments described here might have been under such conditions of moisture stress that their excised shoots were more likely to absorb greater amounts of the toxic metabolite(s) of the bacterium than healthy shoots. This might account for the observed increase in wilt severity of shoots from nematode-infected plants. It is true that occasional wilting of nematode-infected plants occurred, particularly at later stages of nematode infection. It has been pointed out, however, that all the excised shoots were placed in tap water for two to three hours prior to being tested. This allowed any partially wilted shoots to recover full turgidity prior to treatment with the culture filtrates. Thus, it is unlikely that shoots from nematode-infected plants were under any greater water stress than were shoots from healthy plants.

Results of this research offer only the first step toward understanding the nature of the interaction between the nematode and the bacterium. From the evidence presented, it seems that biochemical aspects of the plant-nematode relationship undoubtedly influence and probably govern its interrelationship with the bacterium. The nematode-bacterial interaction represent one of many existing complexes among soil-borne plant pathogens. Steiner (121) has emphasized that nematodes in relation to plants should be considered as members of dynamically interacting biotic entities. Future research work should be directed towards

studying the aspects of the early phases of the interaction of the nematode and the bacterium. Careful histopathological studies to correlate bacterial invasion with nematode penetration will conclusively verify the role of wounding made by the nematode in increasing severity of the bacterial wilt. In conclusion, much research work is needed for elucidating the exact role of the root-knot nematode in predisposing the host to the bacterial wilt.

SUMMARY

Inoculation experiments were conducted in the greenhouse to test the interaction of Meloidogyne incognita acrita and Pseudomonas solanacearum in the wilt-susceptible tomato variety Rutgers and the wilt-resistant tomato breeding line designated as "1961-57-55 MO.P". Experiments also included a study of the comparable effects of the root-knot nematode and artificial root wounding on the incidence and rate of development of the bacterial wilt in both tomato host plants.

Inoculation of tomato seedlings with the bacterium was done immediately after addition of the nematode. Plant roots were artificially wounded prior to inoculation with the bacterium.

Root wounding, infection by the nematode or their combined effects significantly increased the incidence and rate of development of the bacterial wilt in both host plants. The increased rate of bacterial wilt development produced in the presence of root wounding was significantly higher than that produced in the presence of the nematode. Root wounding presumably offered suitable infection courts for massive bacterial invasion within relatively short periods of time prior to wound healing. Since the inoculum of the nematode was introduced as egg masses, hatching of the eggs and release of the infective larvae apparently occurred gradually. Thus, wounds inflicted by the nematode during larval penetration were likely to occur over prolonged periods of time. This, in part, may account for the more rapid increase in the rate of

bacterial wilt development caused by root wounding compared to that produced by the nematode.

At the age of 39 days, plants of the "MO.P" tomato line showed significantly higher resistance to bacterial wilt than did plants of the variety Rutgers. This significant difference in wilt susceptibility occurred even when roots of both host plants were similarly wounded. Since it has been reported that the bacterium readily entered roots of susceptible and wilt-resistant tomato varieties, it is suggested that the resistance of the "MO.P" line is presumably plasmatic in nature.

Laboratory experiments were conducted to test the hypothesis that although wounds inflicted by the nematode might be a contributing factor in predisposing tomato varieties to bacterial wilt, nematode infection somehow alters the physiology of the host so that its susceptibility to bacterial wilt is increased. Excised shoots from nematode-infected and nematode free plants of the wilt-susceptible tomato variety Rutgers and the wilt resistant "MO.P" tomato line were treated with sterile culture filtrate of Pseudomonas solanacearum.

Treatment with the culture filtrate resulted in significantly higher wilt indices of shoots from nematode infected plants than those of shoots from healthy plants of both tomato varieties. This is the first direct evidence supporting the hypothesis above stated. It is therefore concluded that a change in the metabolism of the host in response to the nematode infection is responsible for predisposing susceptible and wilt-resistant tomato varieties to the toxic metabolite(s) of the bacterium. The physiological nature of such me-

tabolic changes was not determined. It is suggested, however, that the nematode directly stimulates bacterial growth by either providing a useful metabolite or by modifying host tissues to a suitable substrate for parasitic establishment of the bacterium and expression of its pathogenic effects.

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BIOGRAPHICAL SKETCH

Mustapha Mabrouk Ben Halim was born February 20, 1934 at Derna, Libya. He attended public high school in Benghazi, Libya. He entered the College of Agriculture at the University of Cairo in September, 1953, and received the degree of Bachelor of Science in Agriculture in June, 1957. In February, 1958 Mr. Ben Halim enrolled in the Graduate School of the University of California, and received the degree of Master of Science in Plant Pathology in June, 1960. From September, 1960, until the present time he pursued his graduate studies toward the degree of Doctor of Philosophy at the University of Florida.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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